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Bursting onto the scene? Exploring stochastic mRNA production in bacteria

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Recent large-scale measurements of gene expression variability (or noise) in *E. coli* have led to the unexpected conclusion that the variability is in large part dictated by and increasing with the mean level of expression. Here we review the evidence for this apparent universal trend in variability, as well as for the related idea that transcription is fundamentally bursty. We examine recently proposed mechanisms for burstiness and universality and argue that they do not explain important features of observed data. Finally, we discuss potential limitations and pitfalls in the interpretation of experimental measurements of cell-to-cell variability.

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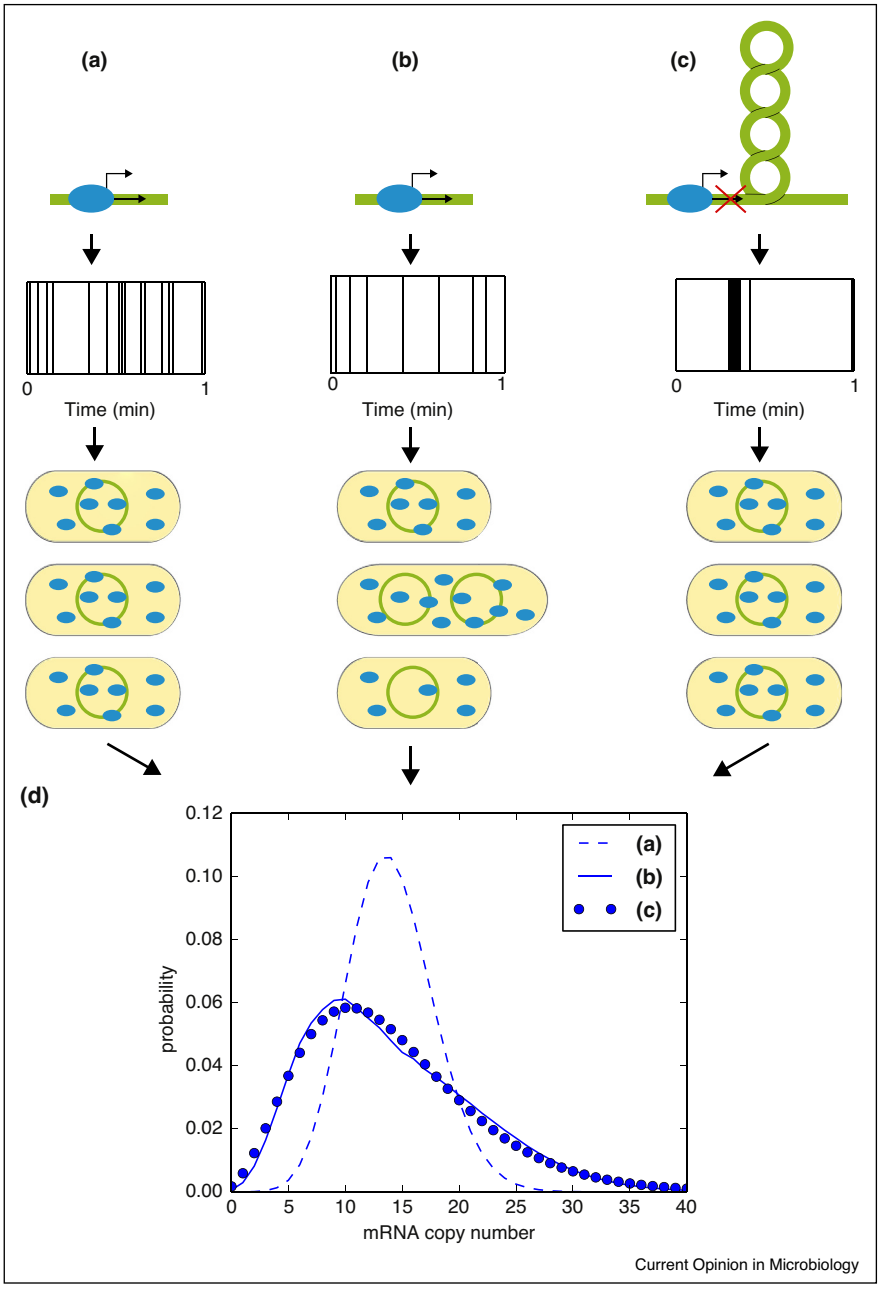
Transcription is the outcome of stochastic single-molecule events such as transcription factor (TF) and RNA polymerase (RNAP) binding, open complex formation, and promoter escape. For this reason, transcription of a given gene even in a genetically identical population of cells will exhibit cell-to-cell variability, or 'noise.' This fact was demonstrated as early as the 1950s in the context of the *lac* promoter [1]. More recently, the results of large-scale studies in *E. coli* have argued for the existence of universal scaling laws in expression variability: that the amount of variability can be predicted simply by knowing the mean expression level [2,3••]. A related but independent idea is that transcription is fundamentally 'bursty'; that is, characterized by extended periods of inactivity punctuated by bursts of transcription, which could under certain assumptions lead to universal scaling in variability [4•]. In this review, we will consider the evidence for universal scaling laws in gene expression variability, as well as proposed mechanisms for transcriptional bursting. Along the way, we will discuss some of the challenges in interpreting data from commonly used experimental techniques in the study of transcriptional variability.

Fundamental modeling and experimental techniques

Well before technologies existed enabling gene expression measurements with single-molecule sensitivity, theorists sought to understand how the distribution of mRNA and protein copy numbers across a population depends on the kinetics of mRNA production [5–7]. A minimalistic model of stochastic mRNA production assumes that a new molecule is transcribed with a constant probability per unit time, that is, in a Poissonian manner, with average rate r, and similarly degraded with rate γ. The resulting distribution is a Poisson distribution with mean and variance equal to r/γ (Figure 1). It has proven useful to characterize distributions in terms of the 'Fano factor,' defined as the variance divided by the mean, which for a Poisson distribution is identically one. The next level of modeling complexity is obtained by allowing the promoter to stochastically switch between 'on' (transcriptionally active) and 'off' (transcriptionally inactive) states [8–11]. This switching increases the level of cell-to-cell variability, leading to Fano factors greater than one. In the limiting case where 'on' periods are short compared to mRNA lifetimes (i.e. mRNA are produced in 'bursts'), mRNA copy numbers follow a negative binomial distribution, with the number of transcripts per burst approximately proportional to the Fano factor (Figure 1). Despite their simplicity, these two models have proved useful as conceptual frameworks for analysis of experimentally measured mRNA distributions [12–14]. Analytical and computational treatments of more complex promoters can be found in [15–19] among others.

In the 2000s, the advent of two important techniques, mRNA FISH and MS2 labeling, made it possible to perform single-cell measurements of transcription with single mRNA sensitivity. Single-cell mRNA FISH (smFISH) enables mRNA counting by labeling mRNA molecules with fluorescently tagged DNA probes complementary to the mRNA [20,21]. The technique is performed in fixed cells and as such provides a 'snapshot' of the gene expression state across a population of cells. Kinetic parameters such as production and degradation rates can be inferred from such snapshots, but doing so is not without certain pitfalls, as we shall

Figure 1



Poissonian vs. bursty mRNA production kinetics. **(a)** A minimalistic model of stochastic mRNA production in which mRNA are produced with Poissonian kinetics. A representative time series of mRNA production events is depicted (middle), with each vertical line representing production of an mRNA. Neglecting cell-to-cell variability in gene copy number and free RNAP concentration (bottom) results in a Poisson distribution across a population of cells, as depicted in (d), dashed line. **(b)** As in (a), mRNA production from individual promoters is Poissonian. However, when accounting for chromosome-replication-driven variation in gene copy number and cell-to-cell variability in RNAP concentration, the resulting distribution is considerably broader ((d), solid line). **(c)** mRNA are produced according to a two-state, 'bursty' model, here depicted (top) as resulting from DNA-supercoiling-dependent transcriptional silencing (although other mechanisms such as the action of TFs could also produce transcriptionally inactive states). The resulting mRNA production events are highly unevenly distributed in time (middle). Neglecting gene copy number and RNAP concentration variations yields a distribution practically indistinguishable from that of (b). **(d)** mRNA copy number distributions resulting from the scenarios depicted in (a), (b), and (c). Parameters were chosen such that all three distributions have the same mean (14 mRNA/cell). In (a), the mRNA production rate $r = 9.3\ \text{min}^{-1}$, and the degradation rate $\gamma = 1/1.5\ \text{min}^{-1}$. In (b), the gene of interest has average copy number 1.4, RNAP concentration varies with 10% coefficient of variation, $r = 6.7\ \text{min}^{-1}$, and $\gamma = 1/1.5\ \text{min}^{-1}$. In (c), mRNA are produced in geometrically distributed bursts of average size 3.1 mRNA, with bursts occurring at rate $3.0\ \text{min}^{-1}$.

see later. The second of these techniques, namely MS2 labeling, entails engineering a series of stem-loops into the 3′ end of the mRNA of interest [22]. Alternatively, a synthetic, non-protein-encoding transcript can be used. The stem-loops are bound by the bacteriophage coat protein MS2 (similar systems have been used with other stem loop-protein pairs), which can in turn be fused to a fluorescent protein, enabling detection of transcripts as they are produced. The advantage of this technique is that it enables real-time observation of transcription events, whereas the disadvantage is that transcripts are effectively 'immortalized' by the presence of the stem loops, disrupting biologically realistic degradation kinetics.

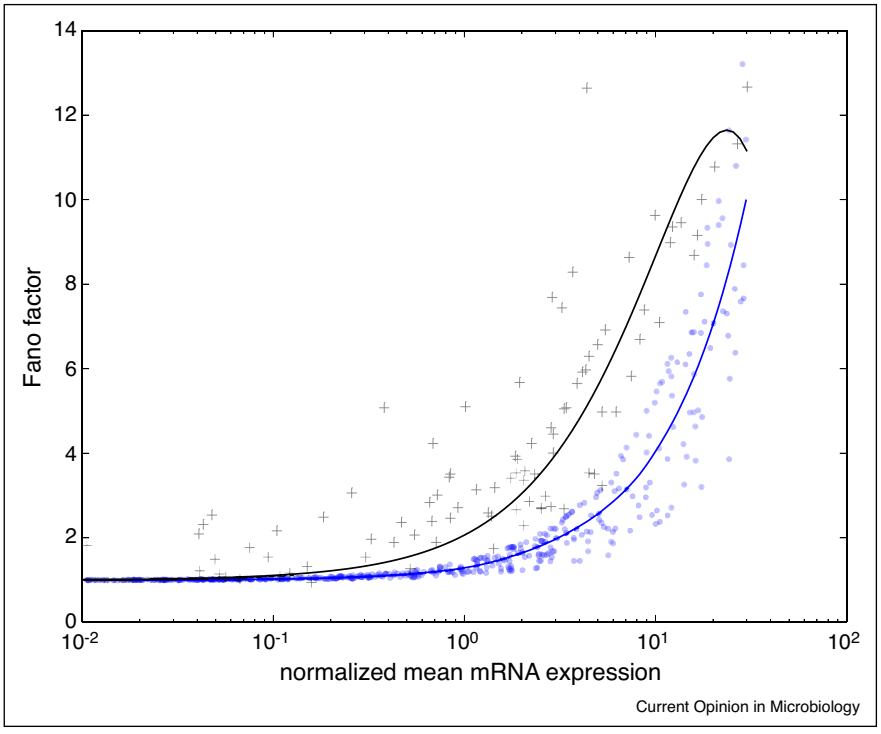
Universal scaling in transcriptional variability

In an influential 2005 study, Golding *et al.* used the MS2 technique to observe production of mRNAs from a hybrid *lac/ara* promoter [23•,24]. Strikingly, they found that, even in the presence of 1 mM IPTG, mRNA production exhibited distinct periods of activity and inactivity, with mean durations of 6 min and 37 min, respectively. Due to the presence of the inducer IPTG, the periods of inactivity could not be attributed to the repressor TF LacI. This observation has been generalized to the idea that transcription is inherently 'bursty': that promoters enter extended periods of inactivity which are not mediated by molecules other than RNAP and the DNA itself [25]. This burstiness increases cell-to-cell variability relative to Poissonian synthesis. Note that the term 'burstiness' has also been used in the context of stochastic gene expression to refer to translation of multiple proteins from a single mRNA, but in this work we restrict our attention to mRNA production.

Subsequent studies in the early 2010s used smFISH to perform large-scale examination of mRNA copy-distributions from a broad range of *E. coli* promoters under various induction conditions [2,3••]. Data from these studies were consistent with the idea of 'bursty' transcription in the sense that the Fano factors of measured distributions were ubiquitously greater than 1, indicating broader-than-Poissonian distributions. Furthermore, So *et al.* argued that the aggregate of Fano-vs-mean data points fall along a universal curve, which is well described by an effective two-state model in which mean gene expression is tuned by altering the rate k_{off} at which the gene enters the transcriptionally inactive state. Similar scalings have been observed in other organisms, reviewed in [14]. They speculated that the observed behavior may be due to changes in DNA topology or 'regulation by broad-target DNA-binding proteins,' possibly analogous to the silencing effect of nucleosomes in eukaryotes. Chong *et al.* subsequently argued that non-TF-mediated silencing was due to the accumulation of RNAP-induced positive supercoils in topologically constrained chromosomal domains, which impede further transcription until released by gyrase. This results in periods of inactivity longer than mRNA lifetimes, and consequently distinct cell subpopulations without mRNA [26,27,28••,29]. However, other studies have observed mRNA distributions from highly expressed genes which lack a subpopulation without mRNA [3••,30••,31•]. Sevier *et al.* later performed a more general mathematical analysis of supercoiling-mediated bursting, without assuming that the timescale of supercoiling release is longer than mRNA lifetimes, and concluded that it could potentially yield variability-vs.-mean curves similar to So *et al.* [4•].

Unfortunately, a recurring challenge in attributing variability to specific causes is the fact that multiple distinct mechanisms can lead to similar variability levels. For instance, changes in gene copy number over the course of the cell cycle lead to a linear increase in Fano factor as a function of mean expression, with a coefficient depending on when in the cell cycle the gene is replicated [30••] (see also [32] for a more detailed and general treatment of this issue). Another potentially meaningful contribution comes from fluctuations in RNAP concentration. It is reasonable to assume that the overall transcription rate will be proportional to the free concentration of RNAP molecules in the cell, and thus fluctuations in free RNAP concentration will also lead to fluctuations in gene expression, yielding again a linear increase in Fano factor with mean expression [30••,32,33]. These factors, often categorized as 'extrinsic noise' [34,35], can create highly non-Poissonian expression distributions even when the underlying kinetics of mRNA production are Poissonian (Figure 1). In fact, gene and RNAP copy number effects alone can partially explain the universal curve of So *et al.* (although in a later work variants of the P_{RM} promoter were observed to lie along the universal curve even after accounting for gene copy variation [31•]). In Figure 2, we simulate a 'toy genome' consisting of 500 constitutively expressed genes, with outputs uniformly distributed in log space between 0.01 and 30 mRNA per gene copy, randomly positioned along the chromosome, with 10% variation in RNAP copy number. At low and very high expression, the Fano vs. mean curve for the toy genome roughly agrees with the universal curve, while lying somewhat below it at intermediate expression. This underestimation is expected since the toy model neglects important sources of variability such as the action of TFs or variability in cell division times. Additional factors that can lead to increased noise include variability in the copy numbers of transcription factors regulating a given gene, or more generally fluctuations in upstream components of regulatory networks [36,37]. Finally, the role of measurement error has been somewhat neglected, but may be worth considering, particularly the potential ambiguity of assigning multiple mRNAs to a single diffraction-limited spot based on intensity.

Figure 2



Gene copy number and RNAP concentration variation can partially account for universal noise scaling. A toy genome of 500 constitutive promoters was simulated, with outputs randomly distributed in log space between 0.01 and 30 mRNA per gene copy, randomly positioned along the chromosome, with 10% variation in RNAP copy number, division time 40 min, C period 51 min, and D period 25 min, chosen to mimic the growth conditions of So *et al.* [3••,38]. The Fano factor of each resulting promoter is plotted vs. its expression per gene copy (blue dots; blue line shows smoothed version). For reference, the data of So *et al.* are also plotted (gray crosses and black summary line). At low and high expression, the Fano vs. mean curve for the toy genome roughly agrees with the universal curve, while lying somewhat below it at intermediate expression.

Variability from constitutive promoters: exceptions to the (universal) rule

In summary, although large-scale experiments measuring mRNA copy number distributions across a range of natural promoters can give a useful feeling for the noise profiles of typical *E. coli* promoters, interpretation of the results in terms of detailed molecular parameters can be challenging. To what extent does observed variability depend on for example, the action of TFs, gene copy number variation, supercoiling-induced silencing, or other factors? To address this question, Jones *et al.* sought to eliminate the effects of TFs by creating a set of 18 constitutive promoters with expression levels ranging from 0.05 to 15 mRNA per cell [30••,39]. These promoters were derived from the *lac* promoter with the LacI and CRP binding sites deleted, such that expression is expected to be unregulated. Expression levels were modulated by changes to the −10 and −35 RNAP binding elements. The researchers found an increasing trend in the Fano factor with increasing gene expression, qualitatively similar to So *et al.* However, they also found that a combination of cell-to-cell variability in gene copy number, estimated RNAP copy number variation, and experimental uncertainty were sufficient to entirely explain the observed deviation from Fano = 1; in other words, that mRNA production from a given constitutive promoter was Poissonian. The largest of these three factors, namely gene copy number variation, could be eliminated by restricting the analysis to either newly divided or about-to-divide cells, which always contain one or two chromosomal copies, respectively. These results seem to argue against a universal role for supercoiling-mediated bursting, although the locus from which expression occurred may have been atypical in some respect (e.g. containing an unusually high density of gyrase binding sites, or falling within an unusually large topologically constrained loop).

In another relevant study, Wolf *et al.* looked at cell-to-cell variability in gene expression from a set of promoters evolved from random sequences to achieve varying levels of constitutive expression via directed evolution [40•]. Their data was acquired at the protein level using relative single-cell fluorescence values from flow cytometry, and therefore the Fano factor cannot be calculated for the absolute mRNA copy number distribution. Nonetheless,

they found that overall the evolved promoters exhibited significantly lower variability in terms of the coefficient of variation than native *E. coli* promoters which were measured simultaneously. Both this work and that of Jones *et al.* demonstrated that it is possible to construct promoters which deviate from universal noise scaling, suggesting that universal scaling stems from sequence-dependent properties of biological promoters rather than being an inevitable byproduct of transcription itself.

Dissecting variability from regulated and biological promoters

Unraveling precisely how features of the promoter sequence dictate cell-to-cell variation (or even mean gene expression levels) is an unsolved problem. Jones *et al.* attempted to take a step in this direction by introducing a binding site for the repressor TF LacI immediately downstream of the promoter for one of their constitutive promoters [30••]. This change in promoter sequence was predicted to introduce an additional, transcriptionally silent promoter state when bound by the repressor. The authors placed LacI under the control of an inducible promoter and found that the variability was consistent with a transcriptionally active state in which mRNA were produced as a Poisson process, and a transcriptionally inactive state, with the fraction of time spent in the respective states determined by LacI concentration. However, Mitarai *et al.* later argued that the data could be better explained by a model explicitly incorporating the multiple kinetic steps of transcription initiation (closed/open complex formation and promoter escape), yielding another example of the difficulty of attributing mechanisms to observed variability-vs-mean curves [41,42].

Moving on to natural promoters, Sepúlveda *et al.* subsequently conducted a detailed, model-based analysis on the maintenance of lysogeny P_{RM} promoter of phage lambda, regulated by the lambda repressor CI [31•]. They expressed a *lacZ* reporter gene from P_{RM} and performed smFISH against the *lacZ* mRNA in fixed cells, while simultaneously measuring CI concentration in each cell using a fluorescent antibody labeling scheme. In this way, they obtained a readout of CI concentration and concomitant mRNA expression in single cells. To explain the observed mRNA distributions, they constructed a model of stochastic mRNA production in which the transition rates between different promoter states (repressed, basal, activated, and looped) depended on the concentration of CI. By fitting the kinetic parameters of the model, they concluded that the promoter switches rapidly (compared to mRNA lifetimes) between the different states. Included in the model was the assumption that, within each of the promoter states, the promoter switches between periods of activity and inactivity. Interestingly, the fitted time between bursts was determined to be as little as half a minute, compared with 37 min in [23•] (see also [12]), suggesting that these observations may not be attributable to a common cause.

It is also interesting to contemplate other modeling choices which may yield equally good fits to the data. The P_{RM} promoter exhibits complex regulation, with a total of six CI binding sites and the ability to adopt looped conformations, resulting in nearly one hundred possible promoter states. In principle, each pair of promoter states has associated kinetic transition rates between the states, yielding a considerable number of fit parameters (even when disallowing unphysical transitions), and each promoter state has a unique associated rate of transcript production. To avoid such a parameter explosion, the authors eliminated states which occur with low probability to winnow down the set to four coarse-grained activity states [43]. It may be, however, that explicitly considering more promoter states without inherent burstiness would yield a comparable quality fit to the data as coarse-grained promoter states with burstiness.

Conclusions

Several aspects of Sepúlveda *et al.* provide a useful template for detailed, model-based analysis of transcriptional variability, such as its deconvolution of gene copy number effects, simultaneous measurement of TF and mRNA copy numbers in single cells, and consideration of full probability distributions rather than summary statistics such as the Fano factor. Nonetheless, there remains in certain respects a divide between studies of simple synthetic promoters and of biological promoters. For instance, we have seen that promoters can be constructed to exhibit varying noise profiles, yet despite interesting hypotheses it remains unsettled which specific features of biological promoters cause them to fall largely along stereotyped or universal curves.

To make progress toward this end, it may be instructive to take a step 'backwards' and look at relatively simple biological promoters which exhibit a more limited set of transcriptional states. Yet, a recurrent theme in this review has been the difficulty of attributing variability to specific sources, as different mechanisms can yield similar variability profiles; for example, different promoter architectures [44], stochastic gene expression and protein partitioning upon cell division [45], or gene copy number and RNAP concentration fluctuations vs bursty mRNA production kinetics (Figure 1). Distinguishing between these cases will require continued careful experimentation in which molecular parameters are isolated and manipulated one at a time to observe their effect on variability, as well as integration of information from orthogonal sources such as measurements of protein binding kinetics and single-molecule experiments.

Conflict of interest statement

Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Novick A, Weiner M: **Enzyme induction as an all-or-none phenomenon**. *Proc Natl Acad Sci U S A* 1957, **43**:553-566.

2. Taniguchi Y, Choi PJ, Li GW, Chen H, Babu M, Hearn J, Emili A, Xie XS: **Quantifying *E. coli* proteome and transcriptome with single-molecule sensitivity in single cells**. *Science* 2010, **329**:533-538.

3. •• So LH, Ghosh A, Zong C, Sepúlveda LA, Segev R, Golding I: **General properties of transcriptional time series in *Escherichia coli***. *Nat Genet* 2011, **43**:554-560.
Measured cell-to-cell variability in mRNA expression from a suite of *E. coli* promoters under varying induction conditions and concluded that the data points in aggregate fall along a universal curve.

4. • Sevier SA, Kessler DA, Levine H: **Mechanical bounds to transcriptional noise**. *Proc Natl Acad Sci U S A* 2016, **113**:13983-13988.
Models the simultaneous effects of TF binding and supercoiling-induced bursting and argues that, in concert, these effects could yield variability vs. mean curves similar to experimentally observed examples.

5. Berg OG: **A model for the statistical fluctuations of protein numbers in a microbial population**. *J Theor Biol* 1978, **71**:587-603.

6. Rigney DR: **Stochastic model of constitutive protein levels in growing and dividing bacterial cells**. *J Theor Biol* 1979, **76**:453-480.

7. McAdams HH, Arkin A: **Stochastic mechanisms in gene expression**. *Proc Natl Acad Sci U S A* 1997, **94**:814-819.

8. Peccoud J, Ycart B: **Markovian modeling of gene product synthesis**. *Theor Popul Biol* 1995, **48**:222-234.

9. Paulsson J: **Models of stochastic gene expression**. *Phys Life Rev* 2005, **2**:157-175.

10. Raj A, Peskin CS, Tranchina D, Vargas DY, Tyagi S: **Stochastic mRNA synthesis in mammalian cells**. *PLoS Biol* 2006, **4**:1707-1719.

11. Shahrezaei V, Swain PS: **Analytical distributions for stochastic gene expression**. *Proc Natl Acad Sci U S A* 2008, **105**:17256-17261.

12. Zong C, So Lh, Sepúlveda LA, Skinner SO, Golding I: **Lysogen stability is determined by the frequency of activity bursts from the fate-determining gene**. *Mol Syst Biol* 2010, **6**:440.

13. Munsky B, Neuert G, van Oudenaarden A: **Using gene expression noise to understand gene regulation**. *Science* 2012, **336**:183-187.

14. Sanchez A, Golding I: **Genetic determinants and cellular constraints in noisy gene expression**. *Science* 2013, **342**:1188-1193.

15. Munsky B, Khammash M: **The finite state projection algorithm for the solution of the chemical master equation**. *J Chem Phys* 2006, **124**:044104.

16. Sanchez A, Garcia HG, Jones D, Phillips R, Kondev J: **Effect of promoter architecture on the cell-to-cell variability in gene expression**. *PLoS Comput Biol* 2011, **7**:e1001100.

17. Neuert G, Munsky B, Tan RZ, Teytelman L, Khammash M, van Oudenaarden A: **Systematic identification of signal-activated stochastic gene regulation**. *Science* 2013, **339**:584-587.

18. Grönlund A, Lötstedt P, Elf J: **Transcription factor binding kinetics constrain noise suppression via negative feedback**. *Nat Commun* 2013, **4**:1864.

19. Munsky B, Fox Z, Neuert G: **Integrating single-molecule experiments and discrete stochastic models to understand heterogeneous gene transcription dynamics**. *Methods* 2015, **85**:12-21.

20. Femino AM, Fay FS, Fogarty K, Singer RH: **Visualization of single RNA transcripts in situ**. *Science* 1998, **280**:585-590.

21. Raj A, van den Bogaard P, Rifkin SA, van Oudenaarden A, Tyagi S: **Imaging individual mRNA molecules using multiple singly labeled probes**. *Nat Methods* 2008, **5**:877-879.

22. Bertrand E, Chartrand P, Schaefer M, Shenoy SM, Singer RH, Long RM: **Localization of ASH1 mRNA particles in living yeast**. *Mol Cell* 1998, **2**:437-445.

23. • Golding I, Paulsson J, Zawilski SM, Cox EC: **Real-time kinetics of gene activity in individual bacteria**. *Cell* 2005, **123**:1025-1036.
Used the MS2 technique to observe transcription events in *E. coli* in real time, and observed periods of transcriptional activity and inactivity ('bursts') which could not be attributed to the action of transcription factors.

24. Lutz R, Bujard H: **Independent and tight regulation of transcriptional units in *Escherichia coli* via the LacR/O, the TetR/O and AraC/I1-I2 regulatory elements**. *Nucleic Acids Res* 1997, **25**:1203-1210.

25. Mitarai N, Dodd IB, Crooks MT, Sneppen K: **The generation of promoter-mediated transcriptional noise in bacteria**. *PLoS Comput Biol* 2008, **4**:e1000109.

26. Liu LF, Wang JC: **Supercoiling of the DNA template during transcription**. *Proc Natl Acad Sci U S A* 1987, **84**:7024-7027.

27. Jeong KS, Ahn J, Khodursky AB: **Spatial patterns of transcriptional activity in the chromosome of *Escherichia coli***. *Genome Biol* 2004, **5**:R86.

28. •• Chong S, Chen C, Ge H, Xie XS: **Mechanism of transcriptional bursting in bacteria**. *Cell* 2014, **158**:314-326.
Demonstrated *in vitro* that transcription-induced supercoiling could stall further transcription, and argued that this mechanism could explain features of observed *in vivo* mRNA expression distributions.

29. Bohrer CH, Roberts E: **A biophysical model of supercoiling dependent transcription predicts a structural aspect to gene regulation**. *BMC Biophys* 2015, **9**:2.

30. •• Jones DL, Brewster RC, Phillips R: **Promoter architecture dictates cell-to-cell variability in gene expression**. *Science* 2014, **346**:1533-1536.
Found that transcription kinetics from rationally designed constitutive promoters were Poissonian, and thus deviated from universal noise scaling. Also analyzed variability from a promoter repressed by LacI using a two-state model.

31. • Sepúlveda LA, Xu H, Zhang J, Wang M, Golding I: **Measurement of gene regulation in individual cells reveals rapid switching between promoter states**. *Science* 2016, **351**:1218-1222.
Experimental investigation of variability from the P_{RM} promoter. Controlled for gene copy number effects and TF copy number variability and used a stochastic model to conclude that the promoter switches rapidly between TF binding configurations and hence different expression states.

32. Peterson JR, Cole JA, Fei J, Ha T, Luthey-Schulten ZA: **Effects of DNA replication on mRNA noise**. *Proc Natl Acad Sci U S A* 2015, **112**:15886-15891.

33. Yang S, Kim S, Lim YR, Kim C, An HJ, Kim Jh, Sung J, Lee NK: **Contribution of RNA polymerase concentration variation to protein expression noise**. *Nat Commun* 2014, **5**:4761.

34. Swain PS, Elowitz MB, Siggia ED: **Intrinsic and extrinsic contributions to stochasticity in gene expression**. *Proc Natl Acad Sci U S A* 2002, **99**:12795-12800.

35. Hilfinger A, Paulsson J: **Separating intrinsic from extrinsic fluctuations in dynamic biological systems**. *Proc Natl Acad Sci U S A* 2011, **108**:12167-12172.

36. Pedraza J, Oudenaarden AV: **Noise propagation in gene networks**. *Science* 2005, **307**:1965-1970.

37. Tkačik G, Walczak AM: **Information transmission in genetic regulatory networks: a review**. *J Phys Condensed Matter* 2011, **23**:153102.

38. Dennis PP, Bremer H: **Modulation of chemical composition and other parameters of the cell at different exponential growth rates**. *EcoSal Plus 2008* 2008 http://dx.doi.org/10.1128/ecosal.5.2.3.

39. Brewster RC, Jones DL, Phillips R: **Tuning promoter strength through RNA polymerase binding site design in *Escherichia coli***. *PLoS Comput Biol* 2012, **8**:e1002811.

40. • Wolf L, Silander OK, van Nimwegen E: **Expression noise facilitates the evolution of gene regulation**. *eLife* 2015, **4**:e05856.

Observed lower variability from constitutive promoters created using directed evolution than from native *E. coli* promoters, and argued that the increase in variability resulting from TF binding sites could be evolutionarily beneficial.

41. McClure WR: **Rate-limiting steps in RNA chain initiation**. *Proc Natl Acad Sci U S A* 1980, **77**:5634-5638.

42. Mitarai N, Semsey S, Sneppen K: **Dynamic competition between transcription initiation and repression: role of nonequilibrium steps in cell-to-cell heterogeneity**. *Phys Rev E* 2015, **92**:022710.

43. Ackers GK, Johnson AD, Shea MA: **Quantitative model for gene regulation by lambda phage repressor**. *Proc Natl Acad Sci U S A* 1982, **79**:1129-1133.

44. Rieckh G, Tkačik G: **Noise and information transmission in promoters with multiple internal states**. *Biophys J* 2014, **106**:1194-1204.

45. Huh D, Paulsson J: **Non-genetic heterogeneity from stochastic partitioning at cell division**. *Nat Genet* 2011, **43**:95-100.